REPLY BRIEF under 37 C.F.R. § 41.41	Attorney Docket No.	3035-101
	First Named Inventor	**Karen Uhlmann**
	Title: **Method of Detecting Epigenetic Biomarkers by Quantitative methylSNP Analysis**	
	Application No.	**10/823,784**
	371(c) Date	April 14, 2004
	Group Art Unit	1634
	Examiner Name	**Amanda Marie Shaw**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

In response to the Examiner's Answer mailed March 30, 2010, applicants submit the following reply.

A request for Oral Hearing is submitted herewith.

The **Status of Claims** is set forth on page 2 of this paper.

The **Grounds of Rejection to be Reviewed on Appeal** are set forth on page 3 of this paper.

Appellants' **Reply Argument** to specific issues raised in the Examiner's Answer starts on page 4 of this paper.

STATUS OF CLAIMS

Rejected Claims:	1 to 5, 7 to 20 and 22 to 39
Allowed Claims:	None
Withdrawn Claims:	None
Claims Objected to:	None
Claims Cancelled:	6 and 21
The appealed claims are:	1 to 5, 7 to 20 and 22 to 39

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The grounds of rejection to be reviewed on appeal are as follows.

A. Whether claims 1-5, 7-9, 11-12, 19-20, 22-24, 26-33 and 36 are obvious under 35 USC §103(a) over Uhlmann et al, CHANGES IN METHYLATION PATTERNS IDENTIFIED BY TWO-DIMENSIONAL DNA FINGERPRINTING, *Electrophoresis* 20: 1748-55 (1999). (hereinafter "Uhlmann '99") in view of U.S. Patent No. 6,258,568 Nyren et al. (hereinafter "Nyren").

B. Whether claims 12-16, 18 and 38 are obvious under 35 USC §103(a) over Uhlmann '99 in view of Nyren, and further in view of US Patent No. 5,786,146 to Herman (hereinafter "Herman").

C. Whether claim 17 is obvious under 35 USC §103(a) over Uhlmann '99 in view of Nyren, and Herman as applied to claims 12 and 38, in further view of US Patent Publication No. 2003/0232351 to Feinberg (hereinafter "Feinberg").

D. Whether claims 10, 25, 34 and 39 are obvious under 35 USC §103(a) over Uhlmann '99 in view of Nyren as applied to claims 1 and 12, and further in view of US Patent 7,078,168 to Sylvan (hereinafter "Sylvan").

E. Whether claim 35 is obvious under 35 USC §103(a) over Uhlmann '99 in view of Nyren as applied to claim 1 and further in view of US Patent Publication No. 2002/0086324 to Laird (hereinafter "Laird").

F. Whether claim 37 is obvious under 35 USC §103(a) over Uhlmann'99 in view of Nyren as applied to claim 1 and 8 and further in view of US Patent 5,602,000 to Hyman (hereinafter "Hyman").

ARGUMENT

All arguments of Appellants' Brief are incorporated herein. However, certain of the points the Examiner raised in the Examiner's Answer are specifically addressed below.

THE COMBINATION OF UHLMANN '99 AND NYREN

On page 15 of the Examiner's Answer, the Examiner responds to appellants' argument that Uhlmann '99 does not disclose the limitation "nucleic acid molecule in an aqueous solution."

The Examiner acknowledged that in Uhlmann '99 the single stranded DNA was "fixed" in agarose beads prior to subjecting the denatured DNA to bisulfite treatment.

However, the Examiner expressed the opinion that since

"the nucleic acid molecule is embedded in an agarose bead and the agarose is in solution of mineral oil, the nucleic acid molecule is **technically** in an aqueous solution (*emphasis added*)."

The Office also refers to Figure 1, where Uhlmann '99 depicts three distinct beads (having single stranded DNA immobilized therein (see legend of Figure 1)) floating in a bisulfite treatment solution to support that Uhlmann '99s' nucleic acid molecules are "in an aqueous solution."

Thus, the Examiner takes the position that as long as there is an "aqueous solution" somewhere proximate to the nucleic acid, even if the nucleic acid is fixated in single stranded form in an agarose bead, the "nucleic acid molecule" is technically "in aqueous solution." The fact that the nucleic acid molecule is immobilized in a distinct agarose bead appears, according to the Examiner's analysis, irrelevant. The Examiner considers her interpretation to be the "broadest reasonable interpretation" (page 15 of the Examiner's Answer, last line).

Appellants note that the broadest reasonable interpretation still needs to be ascertained in light of the specification. In the specification the term "comprising said nucleic acid molecule or consisting of said nucleic acid molecule **in an aqueous solution**" is said to

describe, "the options that the sample may comprise the nucleic acid molecule to be analysed **alone or together with other components that may occur in the neighborhood of the nucleic acid molecule in its natural state** such as components derived from a cell (*emphasis added*)." The specification gives as examples of such other components "RNAs such as rRNA or mRNA when DNA is under investigation or residual genomic or plasmid DNA when RNA is to be analysed." The specification states that care should be taken to remove components from the sample that can interfere with the desired analysis. Later on in the same paragraph, the specification states that the "aqueous solution may be water such as distilled water, a buffered solution such as a phosphate buffered solution or buffered solution other than a phosphate buffered solution, to name some important examples" (see paragraph starting on page 6, line 5 of the present specification).

Appellants respectfully submit that, in view of the above, the Examiner's interpretation of the term "nucleic acid molecule in an aqueous solution" is, given the specification, not consistent with the broadest reasonable interpretation of the term consistent with the specification. Accordingly, Uhlmann '99 does not disclose the limitation "nucleic acid molecule in an aqueous solution."

On page 17, the Examiner states that appellants' argument that an additional step of removing the DNA from the bead would be at odds with the identified advantages (speed etc.) is misleading because even if the step is performed the method of Nyren still has the advantage of enabling a base to be identified in a target position and DNA to be sequenced simply and rapidly by avoiding the need for electrophoresis and use of harmful radiolabels (the Office cites Col. 1, lines 60 to 64 of Nyren).

Appellants respectfully submit their belief that pointing out steps that might need to be performed (or that the person skilled in the art might think need to be performed) when two references are combined is not misleading, when one of the main arguments that the Examiner has provided as motivation why a person skilled in the art would combine the references is speed (see, e.g., reference to "simply and rapidly" in col. 1, line 63 as

cited by the Office). Such a step might discourage the person skilled in the art to make the combination and thus goes to motivation to combine references, which is relevant in an obviousness analysis.

On page 20, the Examiner answers appellant's argument that is based on the fact that Uhlmann '99 only employs the "bisulfite approach" that the Examiner relies on to combine Uhlmann '99 with Nyren to confirm the findings which are the subject of the Uhlmann '99 paper, namely the relation of the methylation status of DNA to the differing melting behavior observed in blood and tumor samples via 2-D fingerprinting. Appellants, which include two of the authors of the Uhlman'99 paper, argued that since Uhlmann '99 tested a theory via the so called "bisulfite approach" (which converts methylated DNA so that it can be distinguished from unmethylated DNA) sought precision, not speed.

The Examiner notes in her answer to this argument that precision is not even mentioned in Uhlmann '99. Appellants concede that Uhlmann '99 does not mention the term "precision."

However, when a theory is to be confirmed with a certain technique, the primary goal, per definition, is generally to confirm the theory, and not to confirm the theory quickly.

Also, the nature of the specific issue posed in Uhlmann '99 strongly suggests that precision would be the primary concern of the authors: A 750bp genomic fragment (including 21 CpG dinucleotides) was analyzed via the bisulfite approach. As it turned out, only one CpG was located in the melting domain of this fragment and this very CpG was found (via the bisulfite approach including subsequent sequencing) methylated in all blood samples, but mostly demethylated in in the tumor sample. The authors could show that this change in methylation status was responsible for the differing fingerprinting patterns that they observed in tumor and blood DNA, respectively, and thus allowed them to conclude that 2-D fingerprinting may be a powerful tool for the detection of DNA methylation changes (see last sentence of abstract).

The "advantages" that the Examiner identified in Nyren to combine its teaching with Uhlmann '99 were thus of minor relevance to the objective of Uhlmann '99.

In addition, considering this high degree of precision that Uhlmann '99 needed, appellants submit that a bisulfite treated sample would aggravate the signal to noise problematic that Nyren discusses in col. 7, lines 48 to 55. In a bisulfite treated sample all cytosine that is unmethylated (up to 95% of the genome) is converted into thymine, which pairs with adenine, a source of dATP, which in turn is, according to Nyren, a major source of background noise. Appellants submit that this background noise could interfere with finding the one methylated CpG on which Uhlmann'99's analysis hinged.

Appellants respectfully submit that there remains overall a gap in the Office's reasoning why a person skilled in the art would combine Uhlmann '99 with Nyren.

The "bisulfite approach" of Uhlmann '99 was chosen to confirm whether the 2D-fingerprinting pattern the authors had observed were indeed the result of differential methylation of tumor and blood DNA. Uhlmann '99 results hinged on the change of the methylation status of one CpG in the melting region of the DNA that could be discovered by sequencing the respective bisulfite treated DNA.
Nyren promises, as reiterated on pages 20 and 21 of the Examiner's Answer, that his sequencing method provides: simplicity and speed, while avoiding the need for electrophoresis and use of harmful radiolabels; the ability to use the method for large scale genome projects or clinical sequencing; automatization and handling of multiple samples at the same time.
According to the Examiner's concluding statement on page 22 of the Examiner's Answer it appears that everybody who wanted to sequence a sample would have chosen the method of Nyren in view of all the advantages that Nyren provides irrespective of the nature of the sample or the particular objective.

Appellants respectfully disagree.

No additional fees are believed to be due with this reply. However, the Commissioner is authorized to charge or debit undersign's deposit account as required.

Respectfully submitted,

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